

# **Standard Methods for Biofilm Testing:**

## **Progress and future directions**

Paul Sturman, Ph.D.  
Industrial Coordinator and Research Professor  
Center for Biofilm Engineering

# Message and Purpose

- Requirements of a standard method
- ASTM biofilm methods
- Adaptability of methods
- Needs for future methods

# Collaboration



EPA

Biological & Economic Analysis Division  
Microbiology Laboratory Branch (MLB)

- Marc Rindal
- Stephen Tomasino
- Charles Odeyale



# Attributes of a standard method:

## The seven R's

- Relevance (lab outcome ~ field outcome)
- Reasonableness (expense, lab techniques)
- Resemblance (controls similar between exps.)
- Repeatability (within lab - denoted  $SD_r$ )
- Ruggedness (unaffected by slight changes)
- Responsiveness (detects changes of interest)
- Reproducibility (among labs – denoted  $SD_R$ )

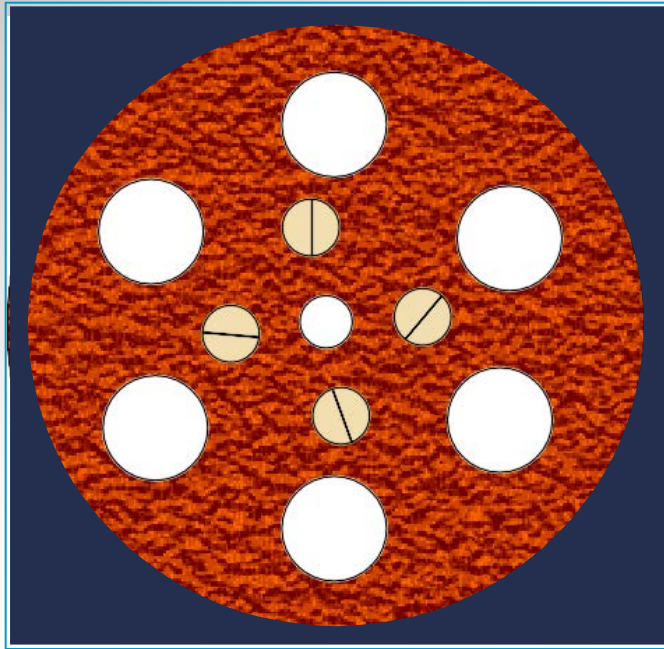
# Attributes of a standard method:

## The seven R's

- **Relevance** (lab outcome ~ field outcome)
- Reasonableness (expense, lab techniques)
- Resemblance (controls similar between exps.)
- **Repeatability** (within lab variation -  $SD_r$ )
- Ruggedness (unaffected by slight changes)
- **Responsiveness** (detects changes of interest)
- **Reproducibility** (var. among labs –  $SD_R$ )

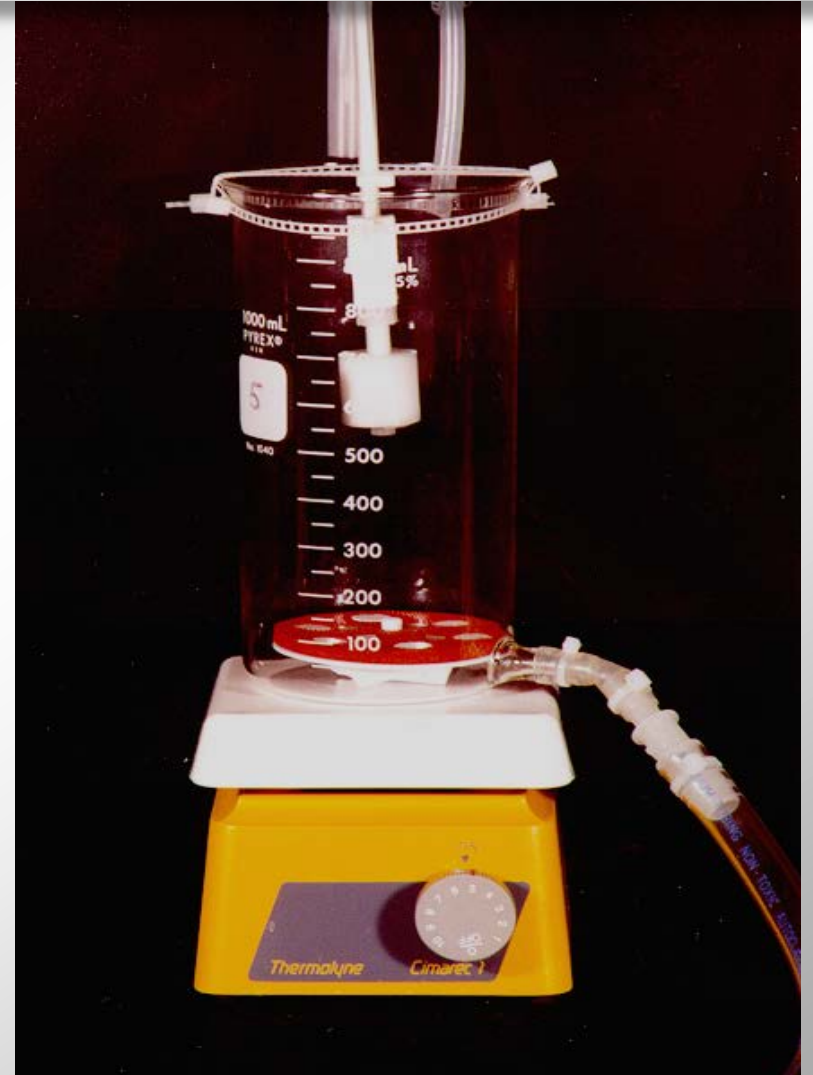


# Relevance: Rotating Disk Reactor as a model toilet bowl

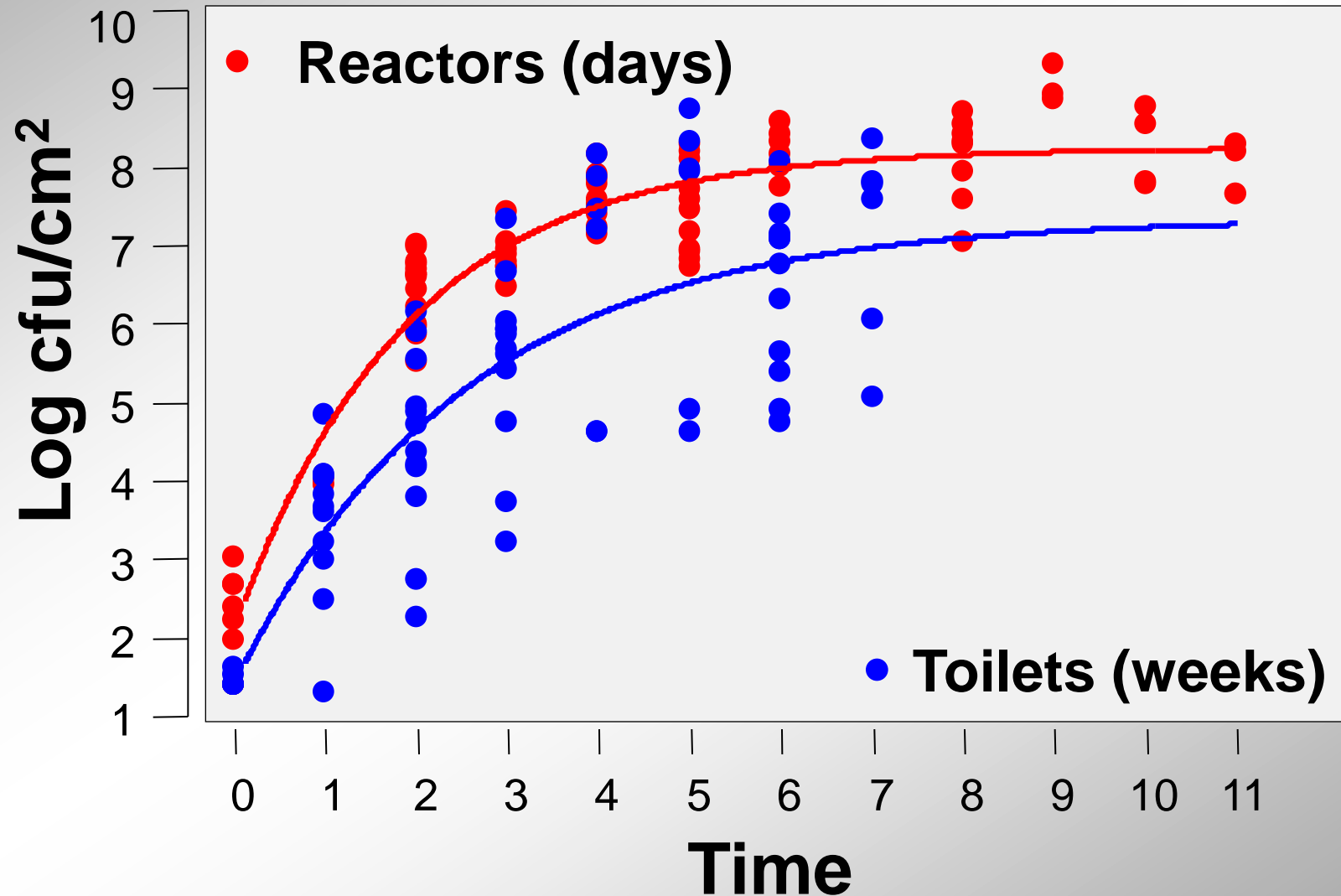


Top

3.0"



# Field validation of biofilm growth method



# Single Tube Method – Inter Laboratory Study Results

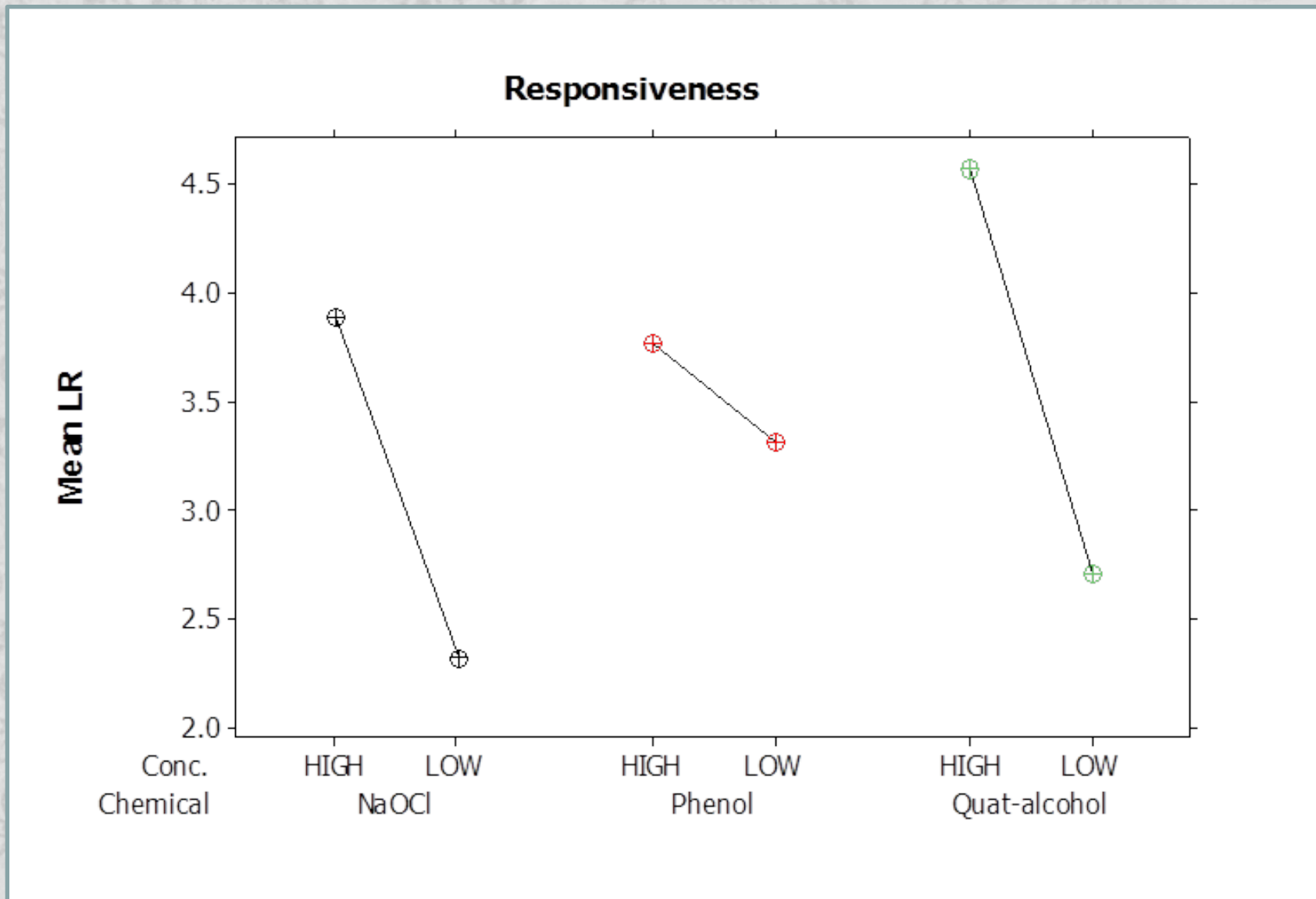
Repeatability

Reproducibility

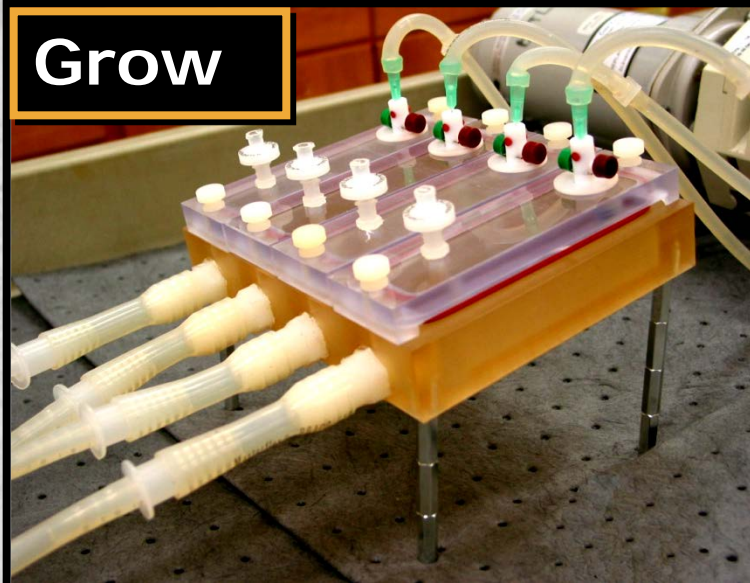
Disinfectant	Conc.	Mean LR	$SD_r$	$SD_R$
Sodium hypochlorite	High	3.89	1.31	1.40
	Low	2.32	0.75	0.89
Phenol	High	3.78	0.58	1.35
	Low	3.32	1.35	1.35
Quat-alcohol	High	4.58	1.45	1.67
	Low	2.71	0.73	0.92



# Responsive to Disinfectant Concentration



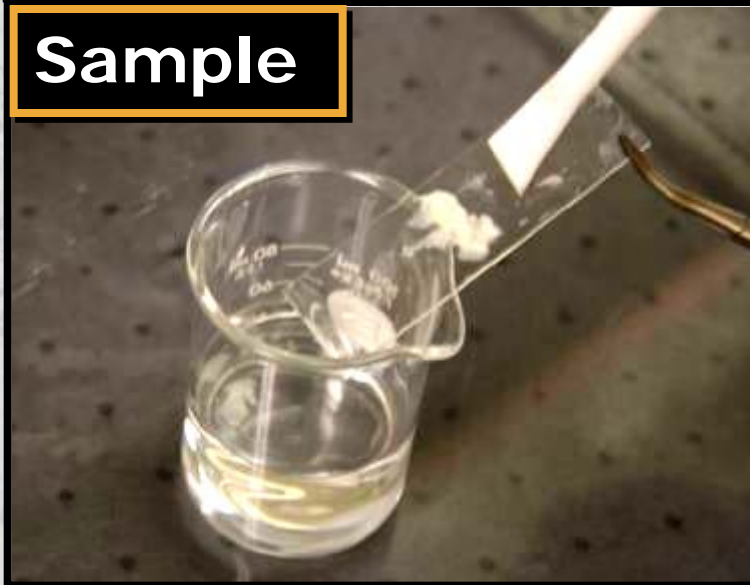
**Grow**



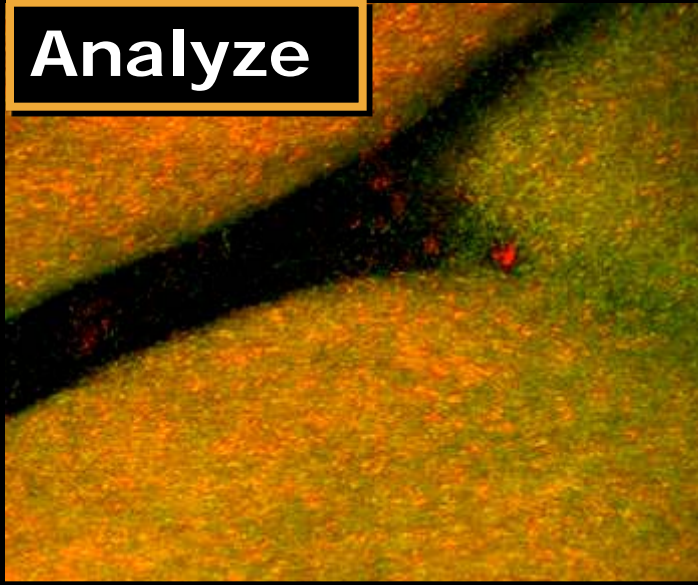
**Treat**



**Sample**



**Analyze**





# Standardized biofilm methods

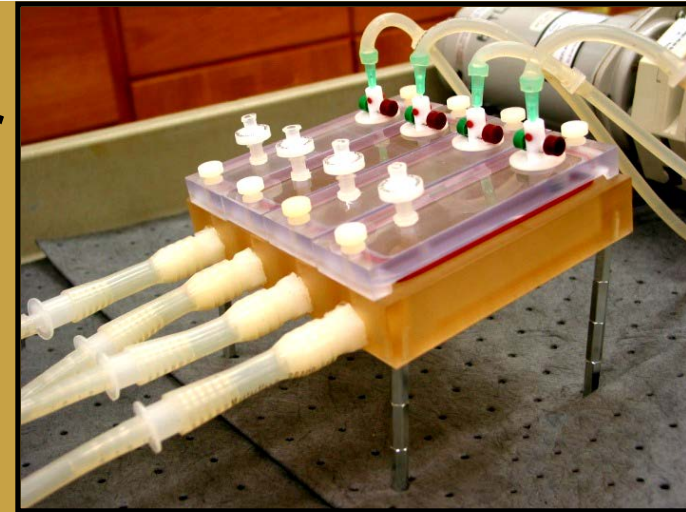


## Rotating Disk Reactor

Moderate shear  
- CSTR -  
ASTM  
Method E2196

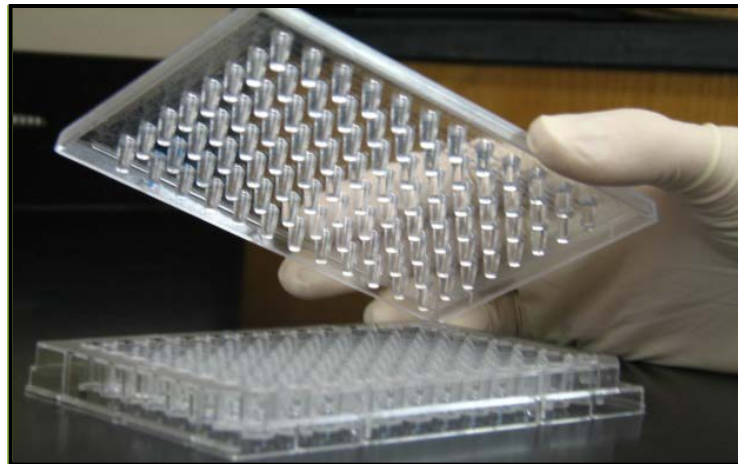
## Drip Flow Biofilm Reactor

Low shear  
- Plug flow -  
ASTM  
Method E2647



## CDC Biofilm Reactor

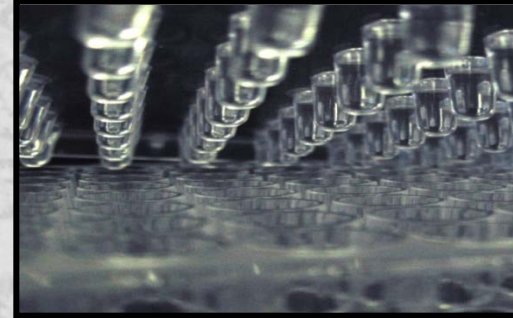
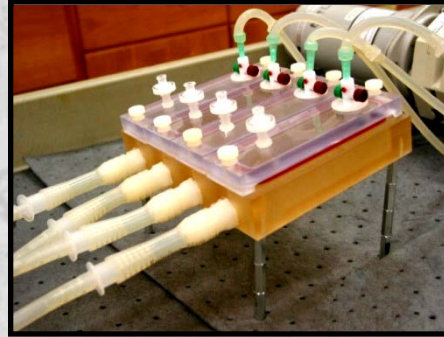
High shear  
- CSTR -  
ASTM  
Method E2562



## MBEC Assay

Gentle shear  
- Batch -  
ASTM  
Method E2799

# ASTM Biofilm Methods Timeline



**2002  
Rotating  
Disk  
Reactor  
E2196**

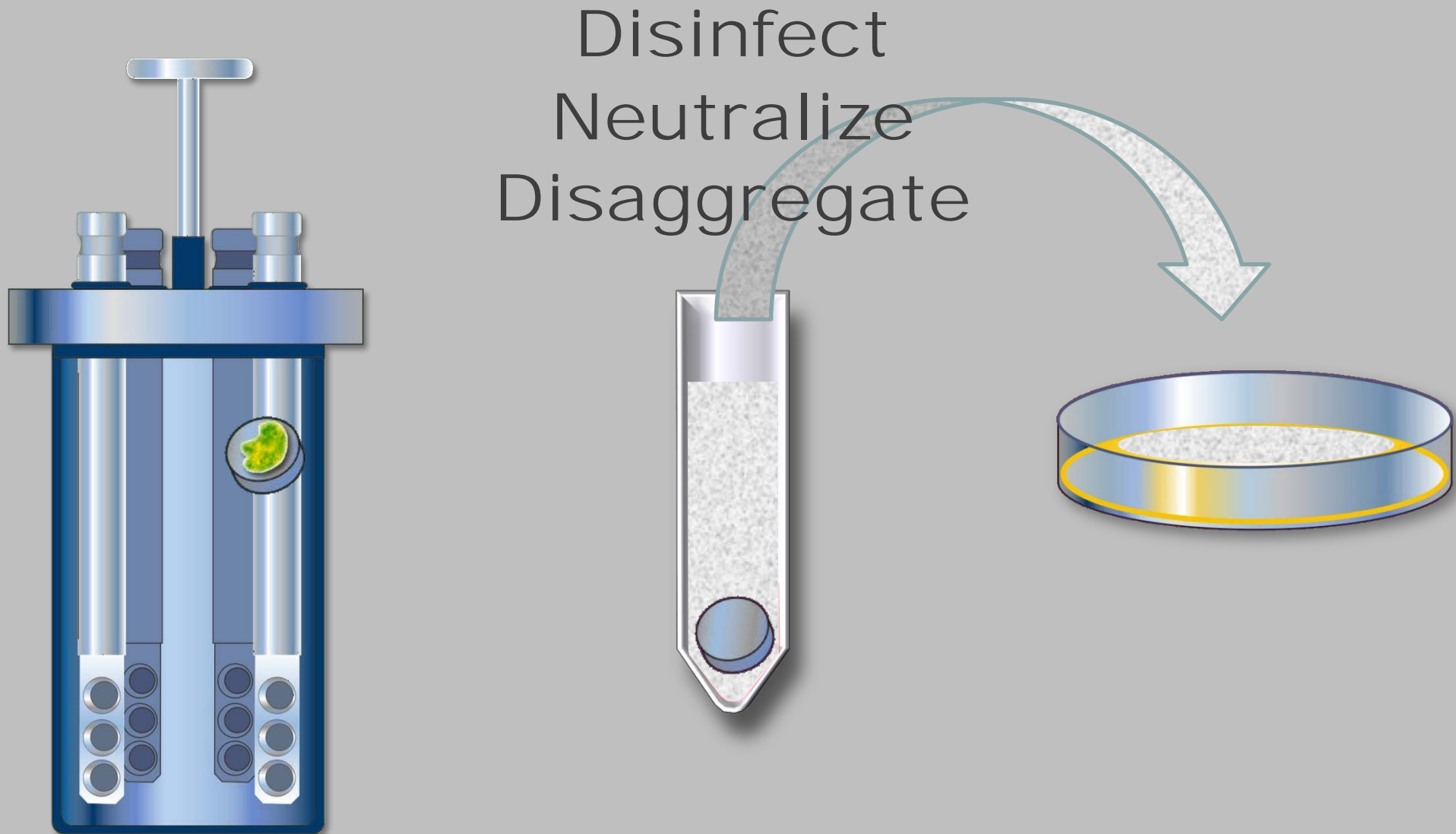
**2007  
CDC  
Reactor  
E2562**

**2008  
Drip  
Flow  
E2647**

**2011  
MBEC  
E2799**

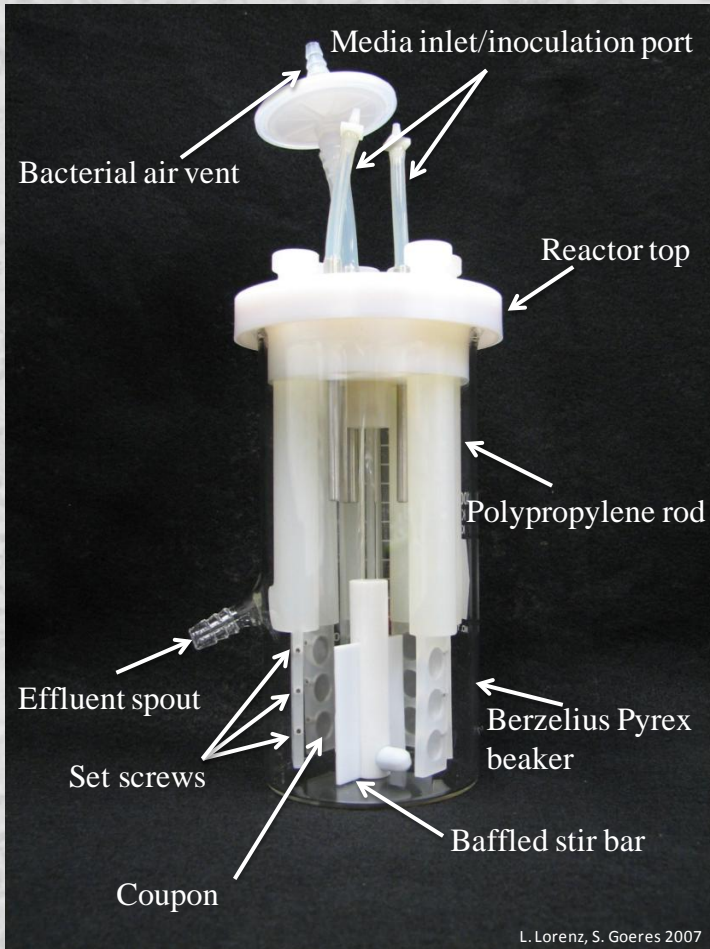
**2013  
Single Tube  
Disinfection  
E2871**

# Single tube disinfection method





# Modifying a Standard Method



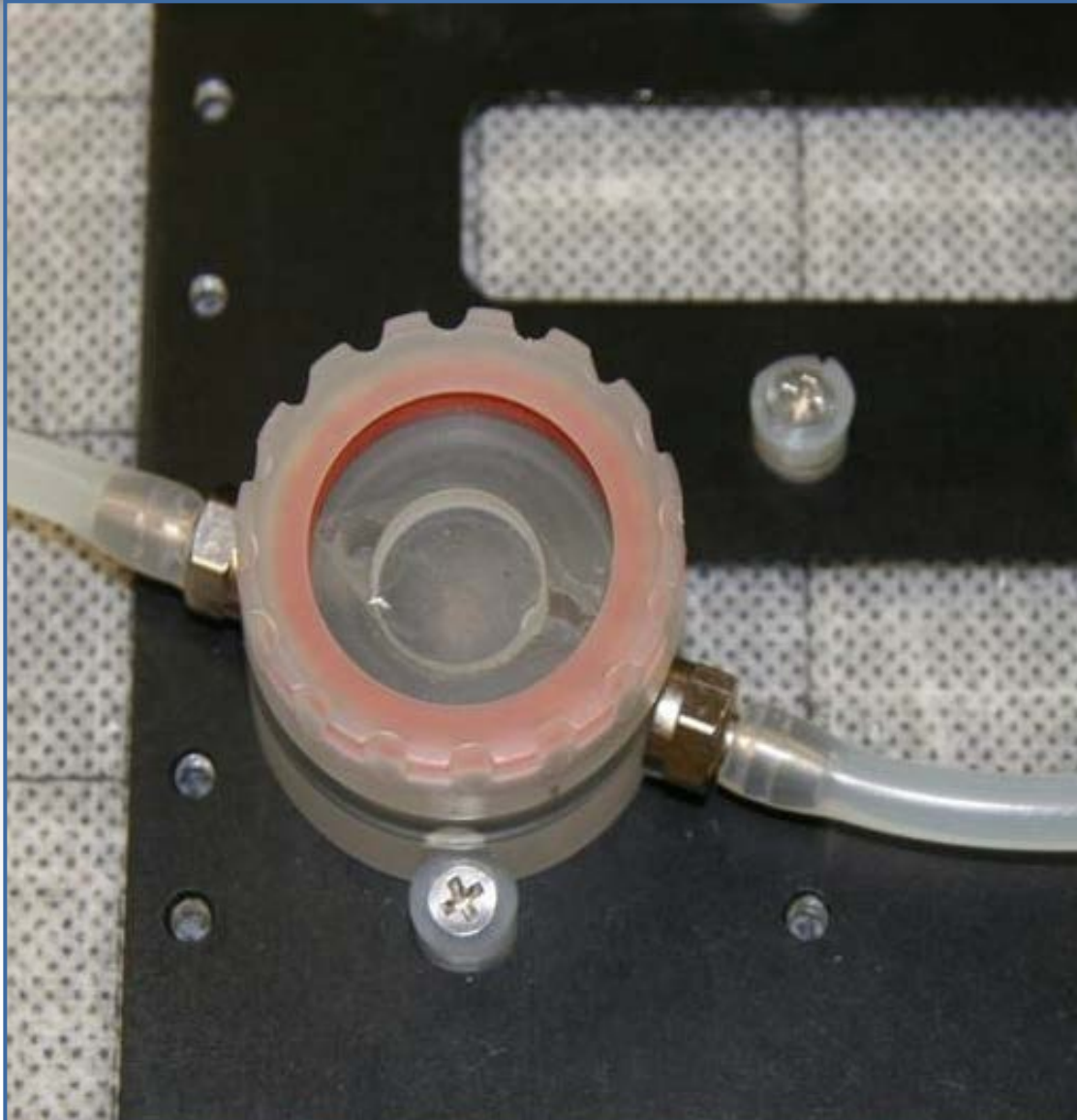
## Potential Adaptations:

- Other species or mixed culture
- Temperature
- Growth Media
- Coupon Surface

# Potential Biofilm Label Claims

- **Reduces** biofilm bacteria (low level of efficacy, for instance 2 logs)
- **Kills** biofilm bacteria (high level of efficacy, for instance 5 logs)
- **Prevents** bacterial biofilm
- **Removes** bacterial biofilm

# Treatment Flow Cell

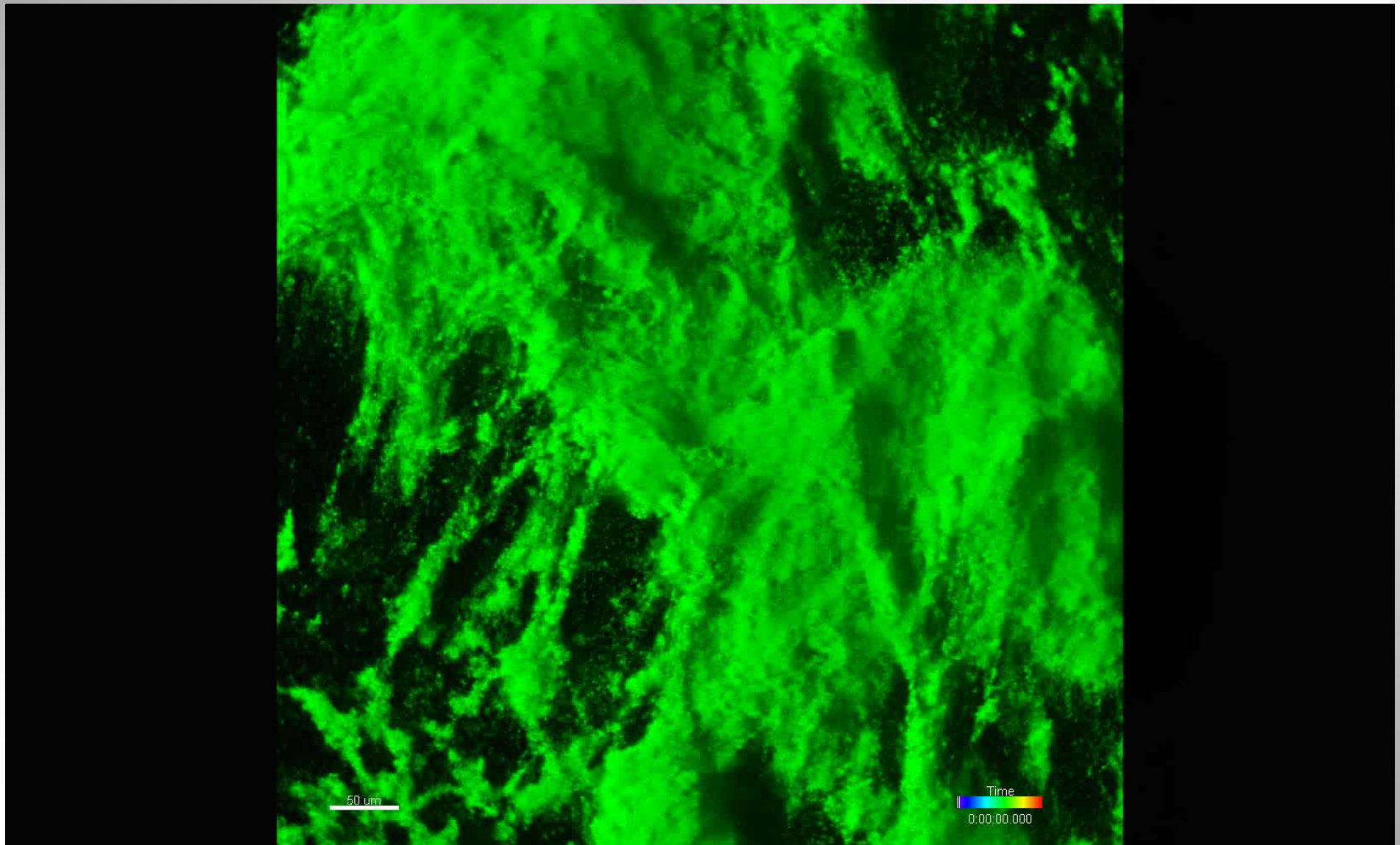


**Advantage:** Real-time, microscopy-based analysis of biofilm accumulation.

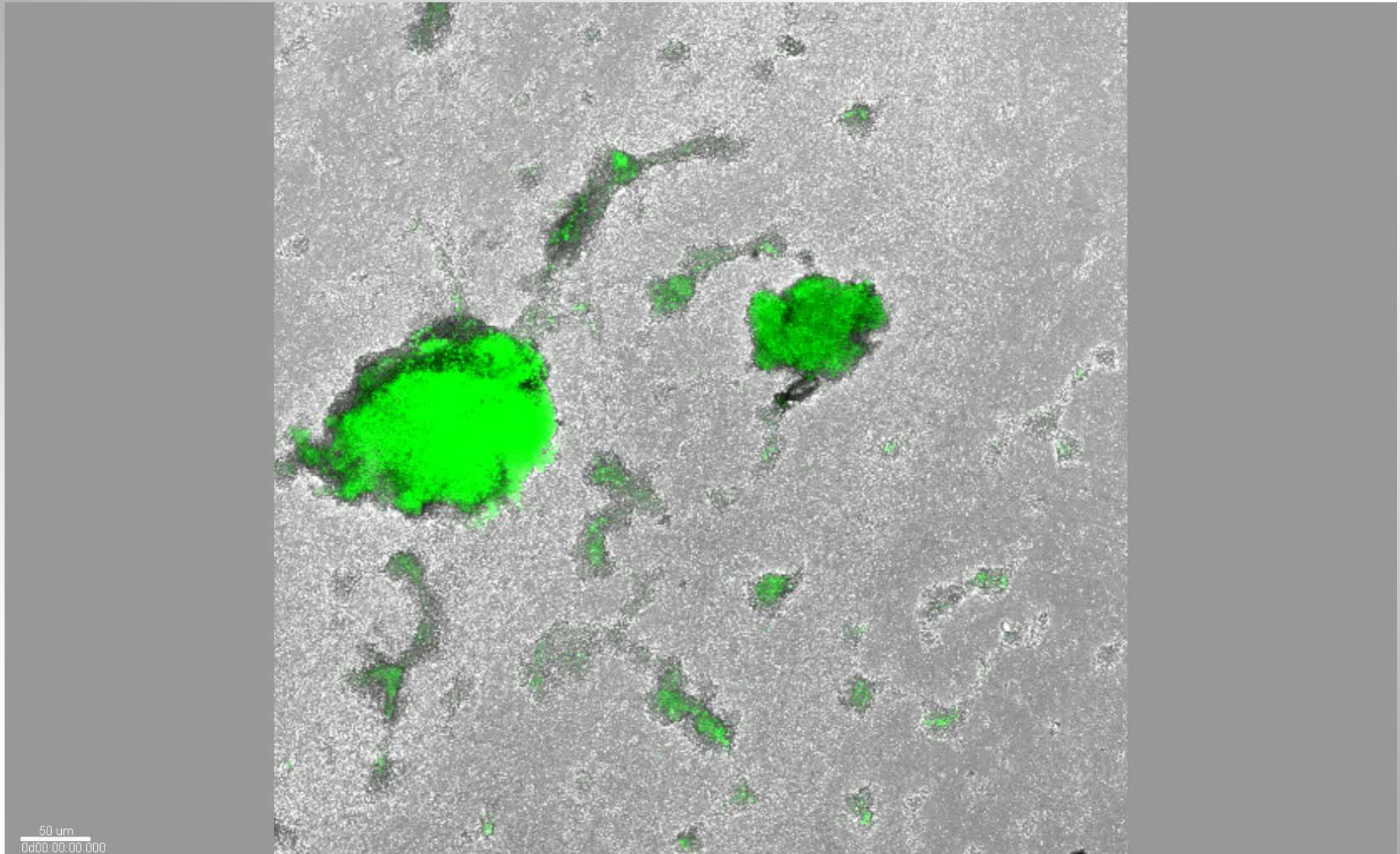
**Limitation:** Must use CDC reactor coupon



# Sodium Bicarbonate in Treatment Flow Cell



# Chlorine in Treatment Flow Cell





# Alternative Removal Assays

- **Microscopy-based Assays**
- **Dry Weight**
- **Total Protein**
- **Total Carbohydrate**
- **Total Organic Carbon**

# Assessing Removal Assays

**Table 1** Summarization of the various factors influencing each quantification protocol.

Compared to SEM ← Accuracy		Repeatability	Time	Cost
XTT	+++	++++	+	++
Crystal Violet	+	++++	++	+
CFU	++	++++	+++	++
DNA	—	+++	++	+++
Amplification	—	++	+++	++++
BCA Protein	+	+	+	+
Dry Cell Weight	—	++	++	+

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## Comparative analysis of *Candida* biofilm quantitation assays

HEATHER T. TAFF\*, JENIEL E. NETT\*<sup>‡</sup> & DAVID R. ANDES\*<sup>†,‡</sup>

Departments of \*Microbiology and Immunology, and <sup>†</sup>Medicine, University of Wisconsin, and <sup>‡</sup>William S. Middleton Veterans Hospital, Wisconsin, USA

# Acknowledgments

- CBE Standard Methods Team
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